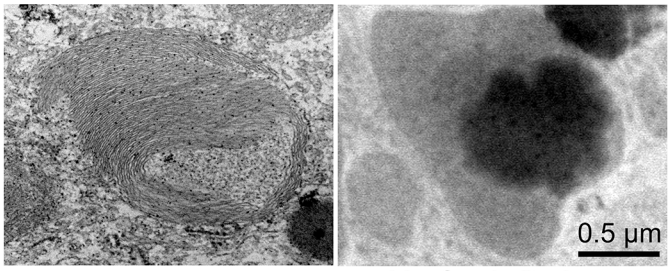
158 New insights in RPE anatomy and physiology
Sunday, May 07, 2017 3:15 PM–5:00 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 1038–1059/B0294–B0315
Organizing Section: Retinal Cell Biology

Program Number: 1038 **Poster Board Number:** B0294
Presentation Time: 3:15 PM–5:00 PM
The role of cAMP and protein kinase A (PKA) in regulation of pigment granule aggregation in RPE of sunfish, *Lepomis spp.*
Christina King-Smith, Joseph Quinlan, Nicole Fischer, Elizabeth Del Rio, Melissa Messalti. Biology, Saint Joseph's University, Philadelphia, PA.
Purpose: Retinomotor movements in eyes of fish and other lower invertebrates include long-distance migration of RPE pigment granules in response to light or dark. Since eyes of these species do not contain dilateable pupils, aggregation and dispersion of pigment granules within long RPE apical projections serves to control light flux to photoreceptors. In dissociated, cultured RPE cells, pigment granule aggregation can be triggered by cAMP. We sought to confirm that cAMP stimulation of aggregation occurs through activation of protein kinase A (PKA), and characterized targets of PKA phosphorylation in RPE to better understand the regulation of pigment granule motility.
Methods: Isolated RPE cells from sunfish (*Lepomis spp*.) were microinjected with PKA catalytic subunit and the position of pigment granules (aggregated vs. dispersed) was compared to un-injected cells. To identify PKA targets, RPE tissue was treated with phosphatase inhibitors and cAMP or dopamine (the chemical trigger for pigment granule dispersion). PKA-phosphorylated proteins were identified by immunoblotting and immunoprecipitation using an anti-Ser/Thr PKA phosphorylated substrate antibody.
Results: Injection of PKA catalytic subunit triggered pigment granule aggregation (N=6 cells) while pigment granules in uninjected cells remained dispersed, demonstrating that cAMP activation of aggregation occurs via PKA activity. Immunoblotting revealed numerous PKA-phosphorylated proteins in cAMP-treated samples; the most abundant were at 112, 82, and 48 kD, in contrast to dopamine-treated RPE cells, which revealed no PKA phosphoproteins via immunoblotting.
Conclusions: While cAMP has been shown to play other roles in cells besides activation of PKA, in fish RPE cells, cAMP elicits pigment granule aggregation via activation of PKA. Previous work has shown that cAMP-triggered pigment granule aggregation involves Rho kinase and non-muscle myosin II. Potential candidates for PKA-phosphorylated targets include proteins associated with the activation of myosin II.
Commercial Relationships: Christina King-Smith, None; **Joseph Quinlan**, None; **Nicole Fischer**, None; **Elizabeth Del Rio**, None; **Melissa Messalti**, None

Program Number: 1039 **Poster Board Number:** B0295
Presentation Time: 3:15 PM–5:00 PM
Remodelling of the basal labyrinth of Retinal Pigment Epithelial cells with osmotic challenge, age and disease
Miguel C. Seabra[2, 1], Matthew Hayes[3], Thomas Burgoyne[3], Silene T. Wavre-Shapton[3], Tanya Tolmachova[1], Clare Futter[3]. [1]Molecular Medicine, Imperial College London, London, United Kingdom; [2]CEDOC, Universidade Nova de Lisboa, Lisbon, Portugal; [3]UCL-Institute of Ophthalmology, London, United Kingdom.
Purpose: The basal surface of the retinal pigment epithelium (RPE) is folded into a complex basal labyrinth. The three dimensional structure of epithelial basal surfaces is thought to affect the transport of solutes and water across them. The structure of the RPE basal labyrinth is perturbed as a result of aging, smoking and in several diseases. The aim of this study was to analyse and define the structural organisation of the basal labyrinth of RPE cells and determine how that structure responds to changes in local osmotic pressure, age and disease.
Methods: We used conventional transmission and serial block-face scanning electron microscopy to examine the structure of the basal labyrinth in eyes from wild type mice and a choroideremia mouse model of different ages with and without osmotic shock before fixation.
Results: We identified structurally distinct zones (*stacked*, *ribbon*-like and *cisternal* regions) within the RPE basal labyrinth. Spacing between the basal infoldings was affected by subtle changes in the osmotic milieu whilst osmotic shock induced dramatic remodelling of the infoldings. Quantitative analysis demonstrated a loss of structure within the basal labyrinth that increased with age and occurred prematurely in a model of choroideremia. A junctional complex crosslinks closely opposed basal infoldings while glycosaminoglycans link more distantly aligned infoldings.
Conclusions: The basal labyrinth is more structured than previously considered, responds to osmotic challenge and is maintained by a number of cross-linking elements. Dramatic and quantifiable changes in architecture of the basal labyrinth occur with age and in choroideremia, which likely compromise trans-epithelial transport.
Commercial Relationships: Miguel C. Seabra; **Matthew Hayes**, None; **Thomas Burgoyne**, None; **Silene T. Wavre-Shapton**, None; **Tanya Tolmachova**, None; **Clare Futter**, None
Support: Wellcome Trust Programme Grant

Program Number: 1040 **Poster Board Number:** B0296
Presentation Time: 3:15 PM–5:00 PM
Multi Lamellar and Lipofuscin Bodies in Aging Monkey Retinal Epithelium
Peter Gouras[1], Lena Ivert[2], Martha Neuringer[3], Takayuki Nagasaki[1]. [1]Ophthalmology, Columbia University, New York, NY; [2]St. Erik's Eye Hospital, Karolinska Institute, Stockholm, Sweden; [3]Oregon Health & Science University, Portland, OR.
Purpose: To determine how the sub-cellular organelles, multi laminar and lipofuscin bodies, change with age in monkey retinal epithelium.
Methods: Retinal epithelium is examined in young and old rhesus monkeys using transmission electron microscopy. Changes in osmium tetroxide fixation was used to distinguish melanosomes from poly-unsaturated fatty acids.
Results: Multi lamellar bodies (MLBs) resemble myeloid bodies found in retinal epithelium usually during the light phase of the circadian cycle. We find them to be common in the aging but not in young monkey retinal epithelium. They can be very large, formed by 50 or more circumferential lamellae. They are normally found in lung epithelium and when seen elsewhere are often a sign of pathology, usually in fatty acid and cholesterol metabolism. In monkey MLBs stratify in the basal third of the retinal epithelium where large lipofuscin bodies are found. Three types off lipofuscin bodies can be distinguished. The most common is a relatively small circular structure with an irregular perimeter and dark particulate electron densities. They stratify in the middle third of the cell. A second type lipofuscin body is larger, has a linear perimeter and contains a lighter electron density, homogenous and centrally placed in the organelle; it stratifies in the basal third of the cell. The third lipofuscin body is rare, has an elliptical or circular shape with a strong electron density; the organelle is located centrally or basally in the cell. If osmium tetroxide was not used, the first two types of lipofuscin bodies lose their electron densities indicating that they contain poly unsaturated

fatty acids. The third type of lipofuscin body keeps its electron density indicating that it contains melanin.
Conclusions: Aging promotes the formation of MLBs and lipofuscin bodies both of which are involved in lipid metabolism and both locate in the basal third of the cell where drusen form from budding plasma membrane.



Commercial Relationships: Peter Gouras, None; **Lena Ivert**, None; **Martha Neuringer**, None; **Takayuki Nagasaki**, None
Support: NIH Grant, Research To Prevent Blindness, Eye Surgery Fund

Program Number: 1041 **Poster Board Number:** B0297
Presentation Time: 3:15 PM–5:00 PM
Automated Quantification of Subcellular Structures in the RPE: Lipid Droplets, Autophagic Vacuoles, and Lipofuscin
Feriel K. Presswalla, Qitao Zhang, David N. Zacks, Debra A. Thompson, Jason Miller. Ophthalmology & Visual Science, University of Michigan Kellogg Eye Center, Ann Arbor, MI.
Purpose: The abundance of subcellular structures changes in response to retinal pigment epithelial (RPE) phagocytosis of photoreceptor outer segments (OS). To quantify these structures in an unbiased way, we developed macros in the ImageJ/Fiji image analysis environment that distinguish and count 3 dissimilar structures: well-circumscribed adipocyte differentiation related protein (ADRP)-marked lipid droplets, indistinct LC3-marked autophagic puncta, and amorphous deposits of lipofuscin that coexist with other sources of spectrally-similar autofluorescence.
Methods: The code for ADRP puncta quantification is based on adjustments to the 3D Spot Segmentation plugin developed by Thomas Boudier. LC3 segmentation was approached with both difference of gaussian (DoG) blurring and Thorsten Wagner's nanoparticle tracking analysis (NTA) background removal tool, which simultaneously reduces homogenous background while reinforcing areas of high-contrast, regardless of absolute local intensity. For lipofuscin, imaging with multiple emission windows enabled separation of lipofuscin from other sources of autofluorescence. Scripts were run on human fetal RPE immunofluorescence images and trends were evaluated against a manualized quantification method.
Results: Our Fiji-based macro quantification of ADRP, LC3, and lipofuscin proved reproducible and accurate compared to manual counts. Further, the total time needed to quantify each image was cut by at least half. Use of a modified LC3 immunofluorescence protocol combining two monoclonal antibodies with optimized permeabilization generated cleaner images and increased analysis accuracy. Separate NTA and DoG scripts each generated LC3 puncta counts, but averaging these counts and then normalizing to the total intensity for each individual image provided the most reproducible assessment of autophagy vacuole number. The accuracy of lipofuscin quantification was improved by masking the autofluoresence of OS adherent to the apical surface of the cultures using indirect immunofluorescence labeling of rhodopsin.
Conclusions: Our customizable ImageJ/Fiji macros can quantify lipid droplets, autophagic vacuoles, and lipofuscin, all important RPE downstream processes of OS phagocytosis, with a higher degree of efficiency and impartiality than can be achieved manually.
Commercial Relationships: Feriel K. Presswalla; **Qitao Zhang**, None; **David N. Zacks**, None; **Debra A. Thompson**, None; **Jason Miller**, None
Support: Research to Prevent Blindness Core Facility Grant, Kellogg Eye Center Pre-Residency Fellowship

Program Number: 1042 **Poster Board Number:** B0298
Presentation Time: 3:15 PM–5:00 PM
CRB2 is involved in the apicobasal polarization of RPE cells by participating in tight junction maintenance and cell cycle arrest
Concepcion Lillo, Alicia Segurado, Vicente Valle, Jorge Fernández-Dolon, Almudena Velasco, Henar Albertos, Antonio E. Paniagua. Cell Biology and Pathology, Institute of Neurosciences of Castilla and León, IBSAL, University of Salamanca, Salamanca, Spain.
Purpose: Apicobasal polarity is essential for epithelial cell function and it is determined by the expression of three polarity protein complexes named Scribble, Par and Crumbs complexes. Scribble complex has been shown to promote basolateral membrane identity while both the Par and the Crumbs complex, promote apical membrane identity. The Crumbs complex is typically composed by PALS1, PATJ and the Crumbs homologs (CRB) proteins. We have recently described the expression of one of these Crumbs proteins named CRB2 in the RPE cells. To better understand this highly regulated process, we have studied the role of the polarity protein, CRB2, in human retinal pigment epithelial (RPE) cells during differentiation *in vitro*.
Methods: To achieve this goal, we have studied the temporal sequence of the establishment of the polarity proteins during human fetal RPE cells polarization. We have then knockdown CRB2 in cultured RPE cells and analyzed their proliferation rate, expression and localization of proteins related with the establishment of junctional complexes and those involved in the acquisition of apicobasal polarization compared to the control RPE cultures. We have finally measured the transepithelial electrical resistance (TER), a direct measure of the strength of the cell-cell junctions, during ordinary differentiation and after a severe disruption of junctional complexes with an acute depletion of Ca^{2+} (calcium switch assay).
Results: We have found that CRB2 is the latest of the entire pull of polarity proteins to be positioned at the cell membrane. Subsequently, the absence of the CRB2 protein from these cells results in a delay in the formation of cell-cell junctions and in an increase in cell proliferation in human RPE cells.
Conclusions: Here, we demonstrate that, in differentiating cultures of RPE cells, CRB2 functions in a late stage in the formation of the junctional complex and apicobasal polarity. Additionally, CRB2 knockdown causes a significant increase in cellular proliferation at the time point when this protein reaches the plasma membrane.
Commercial Relationships: Concepcion Lillo, None; **Alicia Segurado**, None; **Vicente Valle**, None; **Jorge Fernández-Dolon**, None; **Almudena Velasco**, None; **Henar Albertos**, None; **Antonio E. Paniagua**, None

Program Number: 1043 **Poster Board Number:** B0299
Presentation Time: 3:15 PM–5:00 PM
Yap1 is required for maintaining adult RPE differentiation
Qiutang Li[1], Patrick A. Scott[1], Eric Vukmanic[1], Lei Xue[2], Douglas C. Dean[1], Henry J. Kaplan[1], Qingxian Lu[1]. [1]DOVS, University of Louisville, Louisville, KY; [2]School of Life Science and Technology, Tongji University, Shanghai, China.
Purpose: Transcriptional coactivator YAP1 is a major downstream effector of the evolutionarily conserved Hippo pathway that controls organ size by regulating stem cell/progenitor cell proliferation and apoptosis during early development. The importance of YAP1 to the eye was further illustrated by the discovery of the heterozygous mutations in YAP1 causing coloboma in man, and the mutation in YAP1 binding domain of TEAD1 responsible for Sveinsson's chorioretinal atrophy (SCRA). However, YAP1 function in maintenance of RPE phenotype has not been investigated. This study aims to dissect YAP1 function and its regulatory mechanism in RPE cells.
Methods: To directly test the role of YAP1 in the differentiated RPE, we crossed *Yap1* floxed mice with these carrying a Cre recombinase driven by the Best1 promoter, which is activated in the differentiated RPE cells. We investigated the phenotypes progressively developed in the *Yap1*$^{-/-}$ RPE in a timecourse up to 12 months old, using (1) EM for ultrastructure; (2) Immunohistochemistry for markers delineating RPE differentiation status, cell polarity, cell contact, microvilli organization, and cytoskeletal arrangement; (3) RPE barrier assay with fluorescent angiography; and (4) ERG for visual function. Additionally, we also performed the qPCR and Western blot to characterize molecular changes including these in the Wnt/β-catenin signaling pathway.
Results: *Yap1* mutation in differentiated RPE caused diminished tight junctions, cell depolarization and RPE65 loss, retraction of microvilli and loss of pigment. These phenotypes are classic features of epithelial-mesenchymal transition (EMT), which are also evident in RPE during proliferative vitreoretinopathy (PVR). When getting older, the RPE-conditional *Yap1* knockout mice exhibited impaired barrier function of RPE cells, disrupted and diminished choriocapillaris, outer segment loss in the adjacent photoreceptors, and abnormal electrophysiology. The photoreceptors in the conditional knockout mice eventually died between 6-12 months of age. Interestingly, β-catenin expression and its transcriptional activity was significantly increased in the mutant RPE.
Conclusions: YAP1 plays a critical role in maintenance of the RPE differentiating phenotype by downregulation of nuclear β-catenin; and thus, maintaining a constant inhibition of Wnt/β-catenin signaling prevents dedifferentiating EMT in normal adult RPE.
Commercial Relationships: Qiutang Li, None; **Patrick A. Scott**, None; **Eric Vukmanic**, None; **Lei Xue**, None; **Douglas C. Dean**, None; **Henry J. Kaplan**, None; **Qingxian Lu**, None
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Program Number: 1044 **Poster Board Number:** B0300
Presentation Time: 3:15 PM–5:00 PM
Effects of cyclosporine on lysosomal function and autophagy in the Retinal pigment epithelium
Hsuan-Yeh Pan[1], Kristen Hatton[1], Abdulla H. Alamri[2], Mallika Valapala[1]. [1]optometry, Indiana university, Bloomington, IN; [2]college of optometry, state university of New York, New York, NY.
Purpose: RPE is the most active phagocytic cell in the body, phagocytosing 10% of the photoreceptor volume daily. Autophagy, a process involved in recycling of damaged organelles and long-lived proteins is also highly active in the RPE. The terminal events of both phagocytosis and autophagy involve fusion and subsequent degradation in the lysosomes. Dysfunctional lysosomes lead to impaired cellular clearance and subsequent degeneration of the RPE. Transcription Factor EB (TFEB) is identified as a master regulator of lysosomal function and autophagy. A serine/threonine protein phosphatase, calcineurin is known to promote nuclear translocation and activation of TFEB. We investigated the effects of cyclosporine, a calcineurin inhibitor on the TFEB-mediated regulation of lysosomal and autophagy pathway in the RPE.
Methods: ARPE-19 and primary mouse RPE cells were treated with 10 μM cyclosporine for 24 hr. Autophagy was induced by culturing the cells in HBSS for 12-24 hr. Quantitative real time PCR (qPCR) analysis was performed to study the expression of the following genes: Lysosome associated membrane protein 1(LAMP-1), Cathepsin D (CTSD), Mucolipin 1 (MCON1), and ATPase, H+ transporting, lysosomal V0 subunit a1 (ATP6V0A1), BECN1 (Beclin 1), UV radiation resistance-associated gene (UVRAG) and Sequestosome 1 (SQSTM1). Immunoblotting and immunostaining with Microtubule-associated protein 1A/1B-light chain 3 (LC3) antibody was used to study the autophagy pathway. LAMP-1 immunostaining was used to estimate lysosome biogenesis
Results: qPCR analysis of ARPE-19 and primary mouse RPE, showed a downregulation of TFEB-target genes in the autophagy and lysosomal pathway upon treatment with cyclosporine. Starvation-induced autophagy was markedly reduced in cells treated with cyclosporine with concomitant reduction in nuclear levels of TFEB. Reduction in lysosomal number and Cathepsin D activity was also observed upon cyclosporine treatment in cells subjected to starvation.
Conclusions: Our studies suggest that pharmacological inhibition of calcineurin by cyclosporine, decreases TFEB localization and expression of TFEB downstream targets in the RPE. Cyclosporine treatment dampens starvation-induced up-regulation of lysosomal biogenesis and autophagy. Our data suggests that TFEB play a crucial role in maintaining cellular homeostasis in RPE.
Commercial Relationships: Hsuan-Yeh Pan, None; **Kristen Hatton**, None; **Abdulla H. Alamri**, None; **Mallika Valapala**, None

Program Number: 1045 **Poster Board Number:** B0301
Presentation Time: 3:15 PM–5:00 PM
Connexin 43 translocation in retinal pigment epithelium during phagocytosis of photoreceptor outer segments
Julia K. Johansson[1], Teemu Ihalainen[2], Heli Skottman[2], Soile Nymark[1]. [1]BioMediTech, Tampere University of Technology, Tampere, Finland; [2]BioMediTech, University of Tampere, Tampere, Finland.
Purpose: Photoreceptor cells of the retina undergo continuous renewal by constantly generating new outer segment from their base and by shedding the aged outer segment fragments from their distal tips. These fragments are eventually engulfed and digested by the retinal pigment epithelium (RPE) through diurnally coordinated phagocytosis, a process which is still not completely understood. Recent reports have suggested that gap junctions participate in this process, however more detailed studies are missing. We investigated the effect of phagocytosis on connexin 43 (Cx43), the major gap junction protein, localization both in human embryonic stem cell (hESC) derived RPE and mouse RPE.
Methods: The hESC-RPE were spontaneously differentiated and cultured as previously described. Mouse RPE was prepared for immunolabeling by enucleating the eyes and bisecting along the equator. The eyecups were sectioned in Ames' solution buffered with

HEPES. The retina was gently removed from the eyecup leaving the RPE firmly attached to the eyecup preparation.
The phagocytosis was assayed by incubating the purified porcine outer segments on hESC-RPE cultures with and without 18α-glycyrrhetinic acid, a blocker for gap junctions. Phagocytosis was assessed *ex vivo* by preparing the mouse eyes with the retina intact at light onset and 15min, 2h and 10h after it. To detect the bound POS particles, large fields were imaged with Zeiss LSM780 confocal system on inverted Zeiss Cell Observer microscope.
Results: Our results showed that after 10h from light onset, when phagocytosis process is not ongoing, Cx43 localizes to cell-cell junctions as bead-like structures. However, during the first 2h of phagocytosis they were partially translocated from the junctions and also localized around the cell-bound POS particles. Interestingly, when phagocytosis was assayed with 18α-glycyrrhetinic acid, the blocker of gap junctions, we observed a potentiation in the translocation effect.
Conclusions: Our results are consistent with the previous hypothesis that phagocytosis promotes gap junctional disassembly. The data indicates that this occurs through partial translocation from the specific loci in cell-cell junctions to more diffuse distribution in the cell membrane. Furthermore, this translocation was sensitive to the blocking of the gap junctions.
Commercial Relationships: Julia K. Johansson, None; **Teemu Ihalainen**, None; **Heli Skottman**, None; **Soile Nymark**, None
Support: Academy of Finland (grant numbers 294054 and 287287) and Emil Aaltonen Foundation

Program Number: 1047 **Poster Board Number:** B0303
Presentation Time: 3:15 PM–5:00 PM
Regulation of miR-21 expression and exosomes-mediated secretion in human retinal pigmented epithelial cells (HuRPE) exposed to hypoxia
Menaka Thounaojam[1], Diana Gutsaeva[1], Pamela M. Martin[2], Manuela Bartoli[1]. [1]Ophthalmology, Augusta University, Augusta, GA; [2]Biochemistry and Molecular Biology, Augusta University, Augusta, GA.
Purpose: Expression and regulation of microRNAs (miRs) in the retinal tissue is still under investigation. We have identified the retinal pigmented epithelium as an important source of several miRs and as an active producer of the miRs "cargos" exosomes. Here we have studied the mechanisms of production and exosome-mediated release of miR-21, a non-coding small RNA that we have previously studied in the context of its activity in promoting retinal neovascularization and vascular inflammation.
Methods: Primary cultures of human retinal pigmented epithelial cells (HuRPE) and ARPE18 were cultured according to their manufacturer's recommendation. The cells were let polarize for 7-10 days before exposure to hypoxic conditions (pO2= 2+/-0.5) for different times (6, 12 and 24 hours). Exosomes were isolated from the cultured medium by following serial high speed centrifugation. Exosomes yield and identity was confirmed by TEM and gold immunostaining for the specific markers CD65 and flotilin. QPCR was performed to assess miR-21 expression pattern. Angiogenic assays (tube formation, up-regulation of collagenases –MMP2, MMP9) were performed on human retinal endothelial cells (HuREC) exposed to different dilution of isolated exosomes.
Results: Hypoxia significantly increased HuRPE and ARPE18 production of miR-21 and exosomes formation. MiR-21 levels were significantly elevated in exosomes secreted by HuRPE and ARPE18 exposed to hypoxia (peak at 6-12 hours). Exosomes isolated from hypoxic HuRPE and ARPE18 conditioned medium stimulated angiogenic features such as tube formation and MMPs activation in HuREC with highest activity measured at 6 and 12 hours. Same effects were produced by transfecting the cells with miR-21 mimic. Blockade of miR-21 biological effects in cells by transfecting cells with a specific miR-21 inhibitor (a.miR-21), significantly reduced the hypoxic exosomes pro-angiogenic effects.
Conclusions: Our data demonstrate production and exosomes-mediated release of miRs, i.e. miR-21, in HuRPE subjected to pathologically relevant simulants (such as hypoxia/ischemia), thus suggesting a potential role for this mechanism in promoting epigenetic changes as well as fine-tuned regulated gene expression in the diseased retina.
Commercial Relationships: Menaka Thounaojam; **Diana Gutsaeva**, None; **Pamela M. Martin**, None; **Manuela Bartoli**, None
Support: EY022416

Program Number: 1048 **Poster Board Number:** B0304
Presentation Time: 3:15 PM–5:00 PM
Loss of ABCB5 leads to progressive visual loss due to sphingolipid accumulation in Retinal Pigment Epithelium
Yuzuru Sasamoto[1, 2], Gabriel Gonzalez[1, 3], Pallavi Banerjee[2], James D. Akula[2], Vassiliki Poulaki[3], Gretchen Berg[2, 3], Markus H. Frank[2], Bruce Ksander[4], Natasha Frank[1, 3]. [1]Brigham & Women's Hospital, Harvard Medical School, Boston, MA; [2]Boston Children's Hospital, Harvard Medical School, Boston, MA; [3]VA Boston Healthcare System, Boston, MA; [4]Massachusetts Eye and Ear Infirmary, Boston, MA.
Purpose: Retinal pigment epithelium (RPE) has a central role in eye development and is critical for the maintenance of the blood-retinal barrier, and homeostasis and survival of photoreceptors during adult life. Progressive loss of RPE is thought to be a major contributor to age-related macular degeneration (AMD). Currently, the general consensus is that RPE is maintained during aging via proliferation and enlargement of mature adult cells. Recently, however, this idea was challenged when a small population of cells was identified within the human RPE layer that displayed stem cell characteristics *in vitro*. We hypothesize that the stem cell gene ABCB5 is expressed by a subpopulation of RPE cells which retain the ability to self-renew and differentiate and are essential for RPE homeostasis.
Methods: Genetically engineered ABCB5 KO mice and ABCB5 GFP/Cre reporter mice were used to analyze ABCB5 function in retinal maintenance and to monitor murine ABCB5+ RPE cells *in situ*. Mice were evaluated by immunofluorescence, RNA *in situ* hybridization, FACS, and liquid chromatography/mass spectrometry (LC/MS). In addition, *in vivo* evaluation of visual function was performed by comparative full-field electroretinography (ERG) on young and aged ABCB5 KO and ABCB5 WT mice. Two-tailed Student's t-test was used for statistical analysis.
Results: In the adult eye, we found specific GFP (ABCB5) expression in the RPE of ABCB5 GFP/Cre mice, which was further corroborated by ABCB5 mRNA *in situ* hybridization and FACS studies. Moreover, up to 50% of cells within the ABCB5 (+) RPE subpopulation did not express the differentiation marker RPE65. ERG analyses revealed reduction in both the amplitude and the sensitivity of the flash responses, as well as longer implicit times in the one-year-old KO animals compared to age-matched WT mice, indicating that loss of ABCB5 results in progressive functional visual abnormalities. There were associated alterations in lipid metabolism revealed by LC/MS analyses, such as accumulation of pro-apoptotic ceramide and its metabolite GM3 ganglioside.
Conclusions: Our results demonstrate that ABCB5 identifies an RPE65-negative progenitor cell subpopulation within adult RPE.

In addition, we show that intact ABCB5 function is important for normal RPE homeostasis and maturation as demonstrated by its role in sphingolipid metabolism.
Commercial Relationships: Yuzuru Sasamoto; **Gabriel Gonzalez**, NIH/NEI 3R01EY025794-01A1S1 (F); **Pallavi Banerjee**, None; **James D. Akula**, None; **Vassiliki Poulaki**, None; **Gretchen Berg**, None; **Markus H. Frank**, Rheacell GmbH & Co. KG (C), NIH/NEI 3R01EY025794-01A1 (F), Rheacell GmbH & Co. KG (P), NIH/NCI grants R01CA113796, R01CA158467, and R01CA138231 (F), ABCB5-related US patent WO2014130518 A1 (P), Ticeba GmbH (P), Ticeba GmbH (C); **Bruce Ksander**, NIH/NEI 3R01EY025794-01A1 (F), Rheacell GmbH & Co. KG (P), ABCB5-related US patent WO2014130518 A1 (P), Ticeba GmbH (P); **Natasha Frank**, NIH/NEI 3R01EY025794-01A1 (F), Rheacell GmbH & Co. KG (P), ABCB5-related US patent WO2014130518 A1 (P), Ticeba GmbH (P), the Department of Veterans Affairs VA Merit Review Awards VA BLR&D 1I01BX000516 and VA RR&D 1I01RX000989 (F)
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Program Number: 1049 **Poster Board Number:** B0305
Presentation Time: 3:15 PM–5:00 PM
Polarized secretion of pro-inflammatory exosomes by the retinal pigment epithelium is selectively regulated by vitamin A dimers and membrane cholesterol
Aparna Lakkaraju, Colin Germer, Li Xuan Tan, Gulpreet Kaur. Ophthalmology & Visual Sciences, University of Wisconsin-Madison, Madison, WI.
Purpose: Exosomes, vesicles derived from the endolysosomal pathway, participate in intercellular communication and disease processes. Although retinal pigment epithelial (RPE) cells secrete exosomes, little is known about their composition, function and fate. Here, we analyzed the size, morphology and protein repertoires of exosomes released at the apical and basolateral surfaces of polarized primary RPE, and investigated how this is modulated by cholesterol accumulation due to vitamin A dimers.
Methods: Exosomes were purified from the apical and basolateral media of primary porcine RPE cultured on Transwell filters by density gradient ultracentrifugation. Transmission electron microscopy and Nanosight particle tracking were performed to analyze the morphology, size distribution and number of exosomes. Exosomal proteins were analyzed by immunoblotting and mass spectrometry. Live-cell spinning disk microscopy was used to follow internalization of exosomes by RPE cells.
Results: Exosomes secreted by polarized primary RPE have cup-shaped morphologies under TEM and mean diameters of 100 nm. Exosomes released apically have flotillin-1, whereas those released basally have Hsp70. In RPE with the vitamin A dimer A2E, Nanosight tracking showed that significantly more exosomes are released apically compared to controls. We have previously shown that vitamin A dimers increase ceramide levels by abnormally activating acid sphingomyelinase. Ceramide is a cone-shaped lipid that induces inward budding of membranes and increases the biogenesis of exosomes. Immunoblotting and mass spectrometric analysis revealed that exosomes released apically from cells with A2E contain pro-inflammatory proteins. Live-cell imaging showed that naïve RPE monolayers take up RPE-derived exosomes.
Conclusions: Primary adult RPE monolayers secrete exosomes in a polarized manner with a distinct repertoire of proteins packaged into exosomes destined for apical or basolateral release. Vitamin A dimers can promote retinal pathology by inducing secretion of pro-inflammatory exosomes, which can then be taken up by neighboring cells in the retina. This transfer could induce or amplify inflammatory and/or immune responses and spread pathogenic stimuli. Current studies are focused on identifying promising approaches to modulate exosome release and decrease inflammation in the outer retina.
Commercial Relationships: Aparna Lakkaraju, None; **Colin Germer**, None; **Li Xuan Tan**, None; **Gulpreet Kaur**, None
Support: NIH grant R01EY023299, BrightFocus Foundation grant M2015350, Macular Society UK, Research to Prevent Blindness Foundation, Retina Research Foundation Rebecca Meyer Brown Professorship

Program Number: 1050 **Poster Board Number:** B0306
Presentation Time: 3:15 PM–5:00 PM
DHA-NPD1 signaling via cREL downregulates pro-inflammatory Wnt5a in RPE cells
Jorgelina M. Calandria, Khanh Do, Nicolas G. Bazan. Neuroscience Center, LSU Health Sciences Center, New Orleans, LA.
Purpose: Neuroprotectin D1 (NPD1) inhibits the expression of pro-inflammatory genes and enhances the expression of anti-apoptotic proteins of the Bcl-2 family (Mukherjee et al. 2004, PNAS 101:8491) that, along with other translational and post-translational events, become instructive signals that decide RPE cell fate. Wnt5a, a secreted protein from the Wnt family of ligands that promote cell fate specification, was found to be involved in homeostatic circuitry activation of NFkB during innate immune response (Naskar et al, 2014. J Immunol 192:4386). We hypothesized that DHA/NPD1 signaling regulates Wnt5a expression and activity. Thus we studied here NPD1 bioactivity in the regulation of the expression and release of Wnt5a.
Methods: cREL was silenced or overexpressed in human RPE cells, and Wnt5a mRNA was measured using SYBR green real-time PCR assay. The time course of Wnt5a secretion was tested by Western blot in medium and hRPE cells undergoing uncompensated oxidative stress (UOS) triggered by 1200 μM H_2O_2 and 10 ng/ml TNFα, in the presence or absence of NPD1. The role of FZD5 and ROR2 was assessed by silencing the co-receptors and measuring the activity of a NF-κB luciferase reporter.
Results: The silencing of cREL resulted in upregulation of Wnt5a transcription, and the overexpression of the transcription factor elicited the opposite effect, suggesting that NPD1 is regulating Wnt5a via cREL. Wnt5a was released from cells undergoing UOS only in the absence of NPD1. The peak for Wnt5a release was observed at 10 hours after the onset of UOS. NF-κB induction upon addition of Wnt5a was altered by the combination of FZD5 and ROR2 silencing, but not separately. NPD1 selectively halts the increase of FZD5 expression triggered by UOS.
Conclusions: The evidence presented here points to a systemic inflammatory role of Wnt5a in which expression is controlled by NPD1 via cREL. Wnt5a is released and is involved in a positive feedback loop that leads to inflammatory signaling amplification. The mechanism proposed here may be a target of new therapies aiming to decrease proinflammatory processes in diverse conditions, including age-related macular degeneration (AMD).
Commercial Relationships: Jorgelina M. Calandria, None; **Khanh Do**, None; **Nicolas G. Bazan**, None
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Program Number: 1051 **Poster Board Number:** B0307
Presentation Time: 3:15 PM–5:00 PM
The discovery of a new family of lipid mediators, the elovanoids, biosynthesized in human RPE cells
Nicolas G. Bazan[1], Aram Asatryan[1], Pranab K. Mukherjee[1], Rong Yang[2], Nicos Petasis[2], Bokkyoo Jun[1]. [1]Ophthal & Neuroscience, LSU Health New Orleans, New Orleans, LA; [2]Department of Chemistry and Loker Hydrocarbon Research Institute, University of Southern California, Los Angeles, CA.
Purpose: Docosahexaenoic acid (DHA) elongation in the inner segment of photoreceptors leads to synthesis of very-long-chain polyunsaturated fatty acid (VLC-PUFA) omega-3s (n-3) by ELOVL4. Mutations of this enzyme cause certain forms of retinal degenerations. We found that genetic ablation of adiponectin receptor 1 (AdipoR1) results in retinal degeneration (Rice et al., Nature Comm, 2015) preceded by a remarkable downregulation of the VLC-PUFA omega-3 molecular species of phosphatidylcholine. These observations support our previous hypothesis that VLC-PUFA omega-3s are precursors of novel bioactive mediators. Here we report the identification of 32:6,n-3 and of 34:6,n-3 hydroxylated derivatives, their structural confirmation, and their biological activity in retinal pigment epithelial (RPE) cells.
Methods: Human RPE cells were cultured with 32:6 and 34:6. After cells and media were collected, lipids were extracted and loaded onto a liquid chromatography-mass spectrometer (LC-MS/MS) for analysis. We analyzed fatty acids, precursors of elovanoids (27-hydroxy-FA32:6 and 29–hydroxyl-FA34:6), and elovanoids (20,27-dihydroxy-FA32:6 and 22,29-dihydroxy-FA34:6). Elovanoids were synthesized, and matching with deuterium-labeled derivatives was performed.
Results: Both elovanoids and pre-cursors of elovanoids were detected on the cells under oxidative stress. We used m/z 499 -> 93 and 499 -> 401 MRM transitions for 20,27-dihydroxy-FA32:6, and m/z 527 -> 93 and 527 -> 429 transitions for 22,29-dihydroxy-FA34:6 for detection. For corresponding precursors, we used m/z 483 -> 385 for 27-hydroxy-FA32:6, and m/z 511 -> 413 for 29–hydroxyl-FA34:6. For further identification, we performed full fragmentation on elovanoids and found good matches to the standards.
Conclusions: In RPE cells, VLC-PUFAs (32:6,n-3 and 34:6,n-3) produce derivatives through a lipoxygenation step, and we proposed the name elovanoids. We also identified stable hydroxyl-intermediates of their synthesis. Elovanoids also are released to the extracellular space, therefore they might exert autocrine and paracrine bioactivity. Clearly they are remarkable inducers of cell survival under uncompensated oxidative stress conditions. Thus RPE and photoreceptors undergoing uncompensated oxidative stress, might need elovanoids to contribute to sustaining functional integrity.
Commercial Relationships: Nicolas G. Bazan, None; **Aram Asatryan**, None; **Pranab K. Mukherjee**, None; **Rong Yang**, None; **Nicos Petasis**, None; **Bokkyoo Jun**, None
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Program Number: 1052 **Poster Board Number:** B0308
Presentation Time: 3:15 PM–5:00 PM
Characterization of the lipid efflux pathways in RPE
Venkata R. Chavali[1], Fiona La[3], Naqi Haider[1], Daniel J. Rader[2], Nicholas N. Lyssenko[4]. [1]Ophthalmology, University of Pennsylvania, Philadelphia, PA; [2]Department of Genetics, University of Pennsylvania, Philadelphia, PA; [3]School of Engineering and Applied Science, University of Pennsylvania, Philadelphoa, PA; [4]Division of Translational Medicine and Human Genetics, University of Pennsylvania, Philadelphia, PA.
Purpose: The RPE comprises of the outer blood-retinal barrier that is important to the maintenance of lipid homeostasis in the retina. Drusen deposits between the Bruch's membrane and RPE are regarded as the hallmarks of AMD. They are predominantly composed of esterified cholesterol and other lipids, suggesting a role of lipid pathways in the formation of drusen and basal linear deposits. We propose to characterize the complete lipoprotein secretion profile in primary polarized hfRPE and also establish pathways required for the maintenance of basal lipid homeostasis in the RPE.
Methods: Polarized RPE cultures obtained from donor fetal eyes (hfRPE, passage 1-2) were grown on 12 well transwell plates. The hfRPE monolayers were characterized using apical and basal polarity markers, RT-PCR of RPE specific genes and TEER measurement. The media from the apical and basal compartments were collected from hfRPE monolayers after 48 hrs and complete fatty acid and cholesterol profiles were established from both conditioned media fractions using GC-MS and western blot analysis. Cholesterol efflux assays were carried out using [^{3}H] labeled cholesterol, apoA1 and HDL as cholesterol acceptors, T0901317 (LXR agonist) and probucol (ABCA1 inhibitor).
Results: Confluent and well-pigment hfRPE cultures on transwells obtained from 6 different donors eyes were evaluated for their basal fatty acid and cholesterol profiles and cholesterol efflux capacity. The apical/retinal side of hfRPE contained more apoE and cholesterol than the basal/choroid compartment, indicating that the RPE layer released more apoE and cholesterol, likely in apoE-HDL, to the retina. Cholesterol efflux to medium and apoAI was stronger on the basal/choroid side, while efflux to HDL was stronger on the retinal side. Our results suggest that ABCA1 was responsible for efflux to apoAI; while SR-BI, a membrane protein responsible for efflux to HDL.
Conclusions: Our experiments show that the RPE employs several cholesterol efflux pathways and that these pathways are active to different degrees on the choroid and retinal side.
Commercial Relationships: Venkata R. Chavali, None; **Fiona La**, None; **Naqi Haider**, None; **Daniel J. Rader**, None; **Nicholas N. Lyssenko**, None
Support: RPB Funds, Bright Focus Foundation Grant

Program Number: 1053 **Poster Board Number:** B0309
Presentation Time: 3:15 PM–5:00 PM
Defective lysosomal calcium signaling in retinal pigmented epithelium cells from ABCA4$^{-/-}$ mice
Nestor Mas Gomez[1], Wennan Lu[1], Claire H. Mitchell[1, 2]. [1]School of Dental Medicine, University of Pennsylvania, Philadelphia, PA; [2]Ophthalmology, University of Pennsylvania, Philadelphia, PA.
Purpose: Lysosomes are recognized as a major store of intracellular Ca^{2+}, and lysosomal Ca^{2+} signaling is implicated in exocytosis, vesicle fusion, and transcriptional control of autophagy. The TRPML1 cation channel is a major conduit for lysosomal Ca^{2+} efflux and its activity can be compromised by lysosomal lipid accumulation. The lysosomes of RPE cells process the shed tips of photoreceptor outer segments and their own autophagic material, and undegraded

material can accumulate as lipofuscin. RPE cells from the ABCA4$^{-/-}$ mouse model of Stargardt's disease are characterized by large deposit of lipofuscin. Here we asked whether lysosomal Ca^{2+} signaling in the ABCA4$^{-/-}$ mouse was defective
Methods: Mice were treated in accordance with ARVO guidelines. Ca^{2+} levels from isolated RPE cells were determined by Ca^{2+} imaging with dye fura-2; cellular lipofuscin did not affect the dye signal. TRPML1 mRNA was quantified by qPCR. Total lysosomal Ca^{2+} was determined from levels released into the cytoplasm following lysosomal rupture by glycyl-L-phenylalanine-beta-naphthylamide (GPN).
Results: The TRPML1 Ca^{2+} channel was expressed in mouse RPE cells. Stimulation of RPE cells from control mice with channel agonist MLSA1 induced a robust rise in cytoplasmic Ca^{2+}. This Ca^{2+} rise was not dependent on extracellular Ca^{2+} or disrupted by thapsigargin, consistent with its lysosomal source. RPE cells from TRPML1$^{-/-}$ mice did not respond to MLSA1, confirming receptor identification. Finally, ABCA4$^{-/-}$ RPE cells were stimulated with MLSA1 and Ca^{2+} release was decreased by 85% compared to control RPE cells. TRPML1 expression did not differ between RPE cells from control and ABCA4$^{-/-}$ mice. Total lysosomal Ca^{2+} were the same in RPE cells from control and ABCA4$^{-/-}$ mice, as determined with GPN
Conclusions: The release of Ca^{2+} from lysosomes in mouse RPE cells is substantial, and is mediated, at least in part, by the TRPML1 channel. The impaired release of lysosomal Ca^{2+} in RPE cells of the ABCA4$^{-/-}$ mouse may reflect impaired channel activity, as TRPML1 expression and lysosomal Ca^{2+} levels were unchanged. Whether this impaired lysosomal signaling contributes to the pathology in Stargardt's remains to be determined
Commercial Relationships: Nestor Mas Gomez, None; **Wennan Lu**, None; **Claire H. Mitchell**, None
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Program Number: 1054 **Poster Board Number:** B0310
Presentation Time: 3:15 PM–5:00 PM
Effect of Intracellular Junction Formation on VEGF Expression in Retinal Pigment Epithelial Cells
Farhad Farjood, Elizabeth Vargis. Biological Engineering, Utah State University, Logan, UT.
Purpose: Vascular endothelial growth factor (VEGF), a potent angiogenic factor, has been implicated in choroidal neovascularization associated with age-related macular degeneration (AMD). In this work, we investigated the role of cell-cell contact and intracellular junction formation on VEGF expression in cultures of a commercially available human retinal pigment epithelial (RPE) cell line (ARPE-19) and primary human RPE (hRPE) cells.
Methods: Two *in vitro* methods, micropatterning and scratching, were used to control the physical dissociation of RPE cell-cell contact. Micropatterning was achieved using a stencil patterning method. Scratching was performed by scoring monolayers of RPE cells using a pipette tip. Extracellular VEGF expression was assessed using an enzyme-linked immunosorbent assay (ELISA) kit for VEGF. Immunocytochemistry (ICC) was performed to visualize the differential expression of VEGF and a tight junction protein, zonula occludens-1 (ZO-1), in micropatterned and scratched RPE cultures.
Results: Micropatterning RPE cells on different size circular patterns produced varying concentrations of cells with lost cell-cell junctions. Increasing the concentration of cells with fewer intracellular junctions increased extracellular VEGF secretion from both ARPE-19 and hRPE cells. Higher expression of VEGF protein by cells on the edges of patterned and scratched RPE layers was confirmed by ICC. Moreover, removing intracellular junctions by micropatterning and scratching resulted in dislocalization of zonula occludens-1 (ZO-1) from intracellular junctions to the nucleus. The loss of intracellular junction formation and accumulation of ZO-1 in the nucleus correlated with increased VEGF expression.
Conclusions: VEGF expression increases after physical disruption of RPE cell-cell connections. This increase in VEGF expression correlates with both the loss of intracellualr junctions and the localization of ZO-1 in the nucleus of RPE cells.
Commercial Relationships: Farhad Farjood, None; **Elizabeth Vargis**, None
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Program Number: 1055 **Poster Board Number:** B0311
Presentation Time: 3:15 PM–5:00 PM
GHRH/IGF-1 pathway in retinal progenitor cell differentiation
Tsz Kin Ng[1], Jasmine S. Yung[1], Sun On Chan[2], Chi Chiu Wang[2], Herman S. Cheung[3], Andrew V. Schally[3], Chi Pui Pang[1].
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Purpose: Precise retinal cell maturation requires specific signaling pathway to guide retinal progenitor cell differentiation. We previously demonstrated that growth hormone-releasing hormone (GHRH) is expressed in retina and participated in retinal disease development. This study aimed to delineate the biological role of GHRH as well as its downstream signaling factor, insulin-like growth factor-1 (IGF-1), in retinal development and retinal progenitor cell differentiation.
Methods: Retinal GHRH receptor expression was determined by the immunofluorescence analysis on embryonic day (E) 14 to post-natal day (PN) 6 retinas of Sprague-Dawley rats. Retinal progenitor cells from E18 and PN1 rat retinas were isolated, purified and cultured in DMEM/F-12 medium with N2 supplement, basic fibroblast growth factor and epidermal growth factor on the non-adherent culture dishes for 3 days, followed by the treatment of retinal differentiation medium (DMEM/F-12 medium with B27 supplement) with or without the addition of GHRH or IGF-1. Retinal cell specification was evaluated by immunofluorescence analysis of specific retinal cell markers (Brn3b for retinal ganglion cells (RGCs), Rhodopsin for photoreceptors and S100 for Müller glia).
Results: The expression of GHRH receptor was not observed in E14 retina until E18 in the retinal progenitor cell and RGC layer, which was indicated by the immunofluorescence signal of retinal progenitor marker Pax6. GHRH receptor signal persisted to E21 and PN6, and confined in the RGC layer, suggesting its involvement in RGC specification. Increased Brn3b-expressing cells were found in retinal progenitor cell differentiation treatment with GHRH by 3 folds for E18 and 5 folds for PN1 ($p < 0.001$). In contrast, IGF-1 enhanced the occurrence of rhodopsin-expressing cells of E18 retinal progenitor cell differentiation by 10 folds ($p < 0.001$).
Conclusions: GHRH receptor expresses in retinal progenitor cell and RGC layer during retinal development. GHRH promotes the specification of retinal progenitor cells towards retinal ganglion cell lineage, whereas IGF-1 is more prone to the photoreceptor specification.
Commercial Relationships: Tsz Kin Ng, None; **Jasmine S. Yung**, None; **Sun On Chan**, None; **Chi Chiu Wang**, None; **Herman S. Cheung**, None; **Andrew V. Schally**, None; **Chi Pui Pang**, None

Program Number: 1056 **Poster Board Number:** B0312
Presentation Time: 3:15 PM–5:00 PM
CEP290 or IFT88 Loss of Function in RPE Suggest a Role for Primary Cilia in Human iPSC-RPE Maturation and Provide Insights into the Mechanism of Ciliopathy-Induced Retinal Degeneration
Qin Wan[1], Ruchi Sharma[1], Justin Chang[1], Vladimir Khristov[1], Kiyoharu Miyagishima[1], Roba Dejene[1], Quanlong Lu[2], Christopher Westlake[2], Meral Gunay-Aygun[3], Sheldon S. Miller[1], Kapil Bharti[1]. [1]NEI, NIH, Bethesda, MD; [2]NCI, NIH, Bethesda, MD; [3]NHGRI, NIH, Bethesda, MD.
Purpose: Retinal pigment epithelium (RPE), a ciliated monolayer in the back of the eye, is critical for maintaining the health and integrity of adjacent photoreceptors. Our previous studies demonstrate that the primary cilia play important role in the regulation of RPE monolayer maturation. However, a direct genetic link between primary cilium proteins and RPE maturation has not been explored yet. The goal of this study is to determine the role of CEP290 and IFT88, two key primary cilium proteins in RPE development and maturation.
Methods: Healthy or CEP290 mutant human iPSC derived RPE (iPSC-RPE) were grown on semi-permeable transwells to generate a confluent monolayer. Primary cilia in iPSC-RPE were manipulated using doxycycline-inducible IFT88 shRNA lentivirus. The effective knockdown of IFT88 was verified by Western blot. Immunocytochemistry and scanning electron microscopy were used to assess the effect of IFT88 knockdown on primary cilia formation. Gene expression and electrophysiology were used to determine the level of iPSC-RPE monolayer polarization and maturation.
Results: IFT88 knockdown prevented primary cilia formation and yielded sparse apical processes in iPSC-RPE monolayer. In comparison CEP290 mutations led to abnormal primary cilium and apical processes in iPSC-RPE. Gene expression analysis revealed that the RPE-signature genes and adult-specific RPE genes were downregulated in IFT88 knockdown and CEP290 mutant RPE cells. Electrophysiology recordings demonstrated that the suppression of primary cilia with IFT88 shRNA led to significantly decreased RPE transepithelial potential and whole tissue resistance; the electrical responses to physiologically relevant stimuli were also dramatically reduced, indicating deficient RPE maturation and functionality.
Conclusions: Our results demonstrate that IFT88 knockdown and CEP290 mutations severely affected primary cilia formation and profoundly disrupted iPSC-RPE monolayer maturation. This study presents direct evidence for the critical role of primary cilia in RPE maturation, and provides insights into the mechanism of ciliopathy-induced retinal degeneration.
Commercial Relationships: Qin Wan, None; **Ruchi Sharma**, None; **Justin Chang**, None; **Vladimir Khristov**, None; **Kiyoharu Miyagishima**, None; **Roba Dejene**, None; **Quanlong Lu**, None; **Christopher Westlake**, None; **Meral Gunay-Aygun**, None; **Sheldon S. Miller**, None; **Kapil Bharti**, None
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Program Number: 1057 **Poster Board Number:** B0313
Presentation Time: 3:15 PM–5:00 PM
The Effects of Electromagnetic Fields on Cultured Human Retinal Pigment Epithelial Cells
Mozhgan Rezaeikanavi[1], Niyousha Nasrabadi[1], Zahra-Soheila Soheili[2], Abouzar Bagheri[3, 1]. [1]Ocular Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (the Islamic Republic of); [2]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran (the Islamic Republic of); [3]University of Social Welfare and Rehabilitation Sciences, Tehran, Iran (the Islamic Republic of).
Purpose: A great deal of evidence has confirmed that electromagnetic fields can affect the central nervous system and are effective in gene expression as well as development, differentiation, cell proliferation, and apoptosis of embryonic neural cells. This study was conducted to investigate the cellular and molecular changes of cultured human retinal pigment epithelial (hRPE) cells when treated with pulsed electromagnetic fields.
Methods: Cultured neonatal hRPE cells with confluency of 60% were exposed to pulsed electromagnetic field of 1 mT intensity and 50 Hz frequency 8 hours daily for 3 days. In addition to cell proliferation and cell death assays, immunocytochemistry for RPE65, Pax6, Nestin and cytokeratin 8/18 proteins were performed. Extracted RNAs were subjected to real-time PCR for Nestin, Pax6, RPE65, and α-SMA.
Results: Treated hRPE cells did not demonstrate significant change in terms of cell proliferation and cell death. Protein expressions of Pax6, Nestin, and cytokeratin 8/18 were decreased in treated cells compared to controls and remained unchanged for RPE65. Gene expressions of Nestin, RPE65 and Pax6 were decreased in treated cells as compared to controls (P value < 0.05). Gene expression of α-SMA did not reveal a significant change.
Conclusions: Our study demonstrated that both gene and protein expressions of retinal progenitor cells markers were decreased in cultivated hRPE cells after exposure to safe dose of pulsed electromagnetic field. Decreased protein expression of cytokeratin 8/18 and decreased gene expression of RPE65 may be indicative of either transdifferentiation or senescence of RPE cells.
Commercial Relationships: Mozhgan Rezaeikanavi, None; **Niyousha Nasrabadi**, None; **Zahra-Soheila Soheili**, None; **Abouzar Bagheri**, None

Program Number: 1058 **Poster Board Number:** B0314
Presentation Time: 3:15 PM–5:00 PM
Effects of Ranibizumab on gene expressions in ARPE-19 cells during cobalt chloride-mediated hypoxia
Mohammad Riazi Esfahani[1, 2], Zahra Faghiri[1], Mohamed Tarek Moustafa[1], Mohamed Mohamed[1], Abdul Sami Memon[1], Baruch D. Kuppermann[1], Cristina M. Kenney[1]. [1]Ophthalmology, University of California, Irvine, Irvine, CA; [2]Ophthalmology, Tehran University of Medical Sciences, Tehran, Iran (the Islamic Republic of).
Purpose: Hypoxia stimulates synthesis and release of vascular endothelial growth factor (VEGF) that is a key component in the pathogenesis of many retinal disorders. Cobalt chloride (CoCl2) is an iron analogue which can mimic hypoxic conditions when added to the medium. One of the main treatments for those disorders is the monoclonal antibody fragment Ranibizumab (Lucentis), which targets VEGF. However, some controversy exists regarding the effects of Ranibizumab on gene expressions in RPE cells. In this study we evaluated expression levels for pro-angiogenesis, pro-apoptosis and pro-inflammatory genes in RPE cells in response to hypoxia alone and in combination with Ranibizumab.

Methods: Hypoxia was induced in culture of ARPE-19 cells and after 24 hr cell viability was determined using MTT assay. For RNA isolation and qRT-PCR, ARPE-19 cultures were pretreated 24 hr with two concentrations of Ranibizumab (1x and 4x the clinical dose) and then stimulated with 25μM CoCl2 to induce hypoxia. qRT-PCR was used to evaluate the gene expression of VEGF, Bax, CASPASE-3 and IL-33.
Results: 25 μM Cocl2 had no significant effect on the viability of ARPE-19 cells. After treatment with 25 μM CoCl2 there was an increase of CASPASE-3 (3.1 fold, p=0.024) and IL-33 (7.5 fold, p=0.0025) compared to the untreated cultures, but it was reversed for both genes by pretreatment with 1x (CASPASE-3 0.47-fold, p=0.048 & IL-33 0.36-fold, p=0.0041) and 4x Ranibizumab (CASPASE-3 0.49-fold, p=0.046 & IL-33 0.33-fold, p=0.0031) compared to CoCl2 alone.
Pretreatment with 1x Ranibizumab did not significantly change the CoCl2-induced increase in VEGF (3.79-fold, p<0.0001) and BAX (1.78-fold, p=0.0014) gene expressions. Ranibizumab at 4x concentration Significantly increased the VEGF (1.529-fold, p=0.0002) and BAX (1.508-fold, p=0.0007) compared to CoCl2 treatment alone.
Conclusions: Ranibizumab showed some protective effects on apoptosis (Caspase-3) and inflammation (IL-33) at both 1x and 4x, but had either no (1x) or increased (4x) effect on VEGF and BAX gene expressions during CoCL2 -induced hypoxia. Understanding variable effects of Ranibizumab in hypoxia may improve our current understanding of the mechanisms involved in pathogenesis of cell loss after long term use of this treatment in AMD patients.
Commercial Relationships: Mohammad Riazi Esfahani; **Zahra Faghiri**, None; **Mohamed Tarek Moustafa**, None; **Mohamed Mohamed**, None; **Abdul Sami Memon**, None; **Baruch D. Kuppermann**, Regeneron (F), Ophtotech (C), Genentech (R), Alcon (C), Allergan (F), Allergan (R), Regeneron (C), Regeneron (R), Catalyst (C), Genentech (F), Novartis (C), GSK (F), Novartis (R), Genentech (C), Alcon (F), Ophthotech (F), Apellis (F), Allergan (C); **Cristina M. Kenney**, None
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Presentation Time: 3:15 PM–5:00 PM

p53-independent Role of MDM2 in Suppressing NF-κB-mediated Inflammation in Retinal Pigment Epithelium Cells

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Purpose: Our previous studies indicated that Nutlin-3, a MDM2 inhibitor inhibits pathologic retinal endothelial cell proliferation through a p53-dependent mechanism, but the p53-independent functions and effect on retinal pigment epithelial (RPE) cell viability have not been adequately elucidated. LPS induced RPE inflammation via NF-κB is a typical model for endotoxin-induced uveitis. Our current study suggests that Nutlin-3, in a non-toxic, low dosage, significantly inhibited LPS-induced NF-κB activation, independent of p53 function. Here, we sought to investigate the underlying mechanism of the non-canonical, p53-indendepent function of Nutlin-3, as well as the role of MDM2 in ocular inflammation. We hypothesized that MDM2 inhibitors possess an anti-inflammatory function benefiting patients suffering from retinal inflammatory diseases.
Methods: For the *in vitro* experiments, the ARPE-19-NF-κB-luciferase reporter cell line was used to record NF-κB activity during LPS and Nutlin-3 treatment. Cell viability and proliferation was assessed using a cell death ELISA and MTT assays. The downstream pro-inflammatory response of NF-kB was tested by q-PCR or ELISA. NF-κB-GFP and p53 knockout mice were used for the *in vivo* experiments. After LPS intraperitoneal and Nutlin-3 intravitreal injection, ocular inflammation was monitored via H&E staining, focal and full-field ERG, and SD-OCT. The eyes were harvested and subjected for western blotting, q-PCR, immune staining, and ELISA for assessing the anti-inflammatory effect of Nutlin-3.
Results: ARPE-19 NF-kB-luciferase cells were pre-treated with the indicated amounts of Nutlin-3 for 30 minutes to 7 hours, followed by stimulating with LPS (10 ng/ml) for various time intervals. LPS alone dramatically induced NF- κB luciferase reporter gene activity, and Nutlin-3 pre-treatment significantly attenuated LPS-induced response ($p < 0.05$). Inhibition of LPS-induced NF-κB by Nutlin-3 is in both dose- and time-dependent manner. More interestingly, this occured in a p53-independent manner.
Conclusions: Our data suggests that Nutlin-3 has a novel p53-indepenent function in RPE cells by inhibiting LPS mediated NF-κB RPE inflammation. MDM2 inhibitors may have a therapeutic effect in treating patients with uveitis and other retinal inflammatory diseases.
Commercial Relationships: Yan Fan
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